SUGGESTIONS WITH RESPECT TO THE MEASUREMENT OF OSMOTIC PRESSURE

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The cryoscopic method for measuring osmotic pressure in plant tissues is at present the only convenient and probably, if the proper precautions are taken, the only reliable method for determining the osmotic pressure of plant tissues. Some of its advantages and limitations have been discussed by various men, among them Dixon and Atkins (2), and the technique of the operation has been considered by a number of men in this country, particularly by Gortner and Harris (4). While the methods of determining the freezing point of the expressed sap have been fairly well standardized and the necessary precautions have been generally followed by investigators, yet little attention has been given to the methods of freezing the tissue, or to the expression of the sap from tissues. It would be interesting also to have, if possible to obtain, data of osmotic pressures of tissues as determined by the freezing-point method and by the plasmolytic method. Dixon and Atkins have emphasized the necessity of freezing the tissue before expression of the sap, using liquid air to freeze the tissue. Gortner and Harris (4) considered the use of liquid air superfluous, but recently Harvey (5) has found that cabbage leaves, hardened to resist freezing, when frozen at $-5°$ C. yielded a sap with a freezing point of $-1.160°$ C.; when frozen with solid carbon dioxide, $-1.630°$, and when frozen with liquid air, $-1.822°$ C. These differences are great enough to be significant. Harvey does not state specifically what pressures were used. He says that "pressures from 10–30 tons were used on a $2\frac{1}{4}$ inch ram."

In 1916, the authors began some work comparing the plasmolytic and the cryoscopic methods. Various circumstances have prevented thus far any continuation of the work, but the authors feel that the methods for expressing sap, and the relation of the pressure applied to the concentration of the expressed sap, make publication of the results desirable. This paper is concerned with the effect of the temperature at which the tissue is frozen and of the pressure applied on the freezing point of expressed sap. The paper includes also data on the osmotic pressures as determined by the plasmolytic and cryoscopic methods; a special apparatus is described for use in expressing sap, and suggestions are made for applying pressures of known values in expressing the sap.

METHODS

In the experiment here reported, two different species of plants were used: *Zebrina pendula* Schnizl, and *Iresine Herbstii* Hook. These plants

were employed because a large supply was at hand and all were growing under the same environmental conditions and the plants of each species were of equal age. Furthermore, the presence of pigments in the cell sap makes easier plasmolytic determinations.

Plasmolytic determinations were made of the pigmented mesophyll cells of Iresine and of the pigmented cells of the lower epidermis of Zebrina. In making the determinations, free-hand cross sections of the leaves were used.

In the plasmolytic determinations, two solutions were used: calcium chloride and sucrose. Calcium chloride was used in preference to other salts for the reason that permeability, as shown by Osterhout (9, 10), True and Bartlett (12), and others, is generally decreased by calcium chloride. No correction was made for shrinkage. Shrinkage of cells would tend to make the osmotic pressure determination higher than the actual. Renner (11) believes that values obtained by the plasmolytic method would generally be lower than those obtained by the cryoscopic method, since in the latter, calculations are based on weight-normal solutions (gram molecules of solute per 1,000 grams of solvent), while in the plasmolytic method one generally uses volume-normal solutions (gram molecules of solute in 1,000 grams of solution). Throughout the plasmolytic experiments weight-normal solutions were used. Therefore, the values obtained by each method should be more comparable.

The osmotic-pressure values by the plasmolytic method were calculated, using 22.4 atmospheres as the osmotic pressure exerted by a weight-normal solution, and, for the cryoscopic method, osmotic pressure $= \Delta \times 22.4/1.86$. It might be more desirable to use the osmotic-pressure values obtained by Morse [see Findlay (3)] and his co-workers, but the values would be only slightly increased. In calculating osmotic pressures from the plasmolytic determinations made with calcium chloride, the formulas given by Livingston (8) were used. Dissociation was calculated from conductivity tables in Landolt-Börnstein (7).

All determinations were made of the leaves only. The plants were cut at 8 A.M. and placed with their cut ends in water for thirty minutes, so that the leaves would be in a turgid condition. In making the plasmolytic determinations, preliminary studies were previously made in order to obtain the approximate concentrations which would be isosmotic with the cell sap, and then, before each series of determinations, various dilutions of the two solutions were prepared, the concentrations of these ranging close to the anticipated threshold solution. In this way rapidity in determining the isosmotic concentration was obtained.

In expressing the sap for the cryoscopic method, the leaves were frozen either in salt-ice mixture or in liquid air. For the liquid-air treatment the leaves were strung on a thread and immersed in a Dewar flask containing the liquid air. The leaves were removed when the liquid air stopped boiling, indicating that the leaves had assumed the temperature of liquid air.

The leaves when removed were very brittle, and they were immediately placed in a wide-mouthed glass-stoppered bottle in order to prevent condensation of water on their surfaces. In freezing the leaves with ice-salt mixture, the leaves were first placed in a glass-stoppered wide-mouthed bottle and then immersed in the freezing mixture until the leaves were frozen solid. The time of exposure was one hour. The bottle was then removed and carefully rinsed to free the outside surfaces of any adhering salt, and then wiped dry. The amount of leaves used in every case was 50 grams of fresh leaves, which for Zebrina is approximately 125 leaves and for Iresine 250 leaves.

For expressing the sap a special apparatus was constructed (fig. 1). This consists of a steel cylinder, internal diameter four inches, and a closely

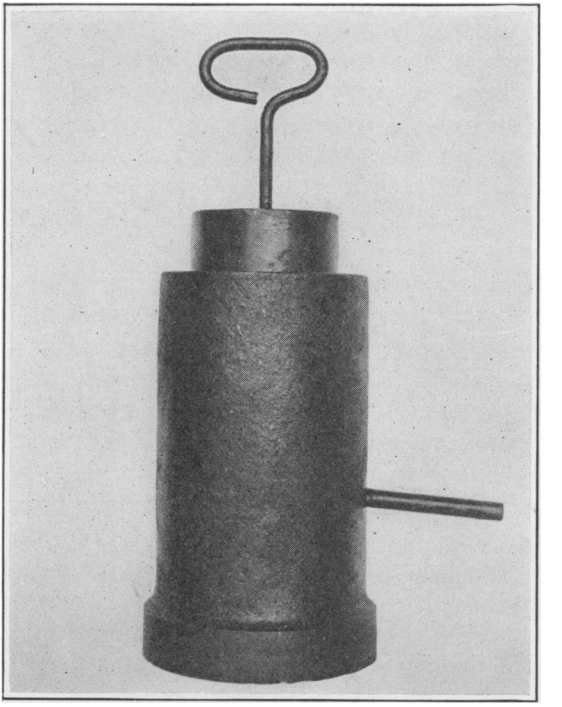

FIG. 1. Apparatus for expression of sap. The handle is unscrewed before the apparatus is placed in the press.

fitting solid steel piston. At the base of the interior of the cylinder, around the circumference, is a small groove 1/16 inch deep and 1/16 inch wide. There is an opening from this groove leading to the exterior, which is fitted with a steel tube of a diameter of 1/8 inch. The purpose of the groove is to prevent the juice from being forced upwards between the cylinder and the piston and also to prevent the tissue from being forced outwards through

the tube. The leaves were wrapped in several layers of washed muslin before being placed in the cylinder. The cylinder and piston apparatus may, of course, be constructed of any size which would be convenient.

For applying pressure to the piston, use was made of materials-testing machinery of the College of Engineering. The one used gave a total pressure up to 50,000 pounds, while with the others available, pressures up to 400,000 pounds could be obtained. The advantage of these machines is that absolutely known pressures can be obtained, and they are available in all engineering laboratories.

The freezing points were determined by the Beckman apparatus. The sap was allowed to undercool to $-1.0°$ C., when it was inoculated by means of a platinum needle with crystals of hoar frost. Undercooling, therefore, was the same in all cases and practically negligible as to its influence.

Experiments

In this experiment, the influence of the method of freezing and of the amount of pressure applied to the tissue is noted. Leaves of Iresine were used. Total pressures of 50,000 and 10,000 pounds were used, and the tissue was frozen either by liquid air or by the salt-ice mixture. The data given in table 1 record the osmotic pressures as calculated from the depression of the freezing point.

Table 1

Method of Freezing	Pressure Applied to Leaves	Osmotic Pressure in Atmospheres
Ice and salt	50,000 lbs.	5.674
Liquid air	50,000 lbs.	5.714
Ice and salt	10,000 lbs.	4.571
Liquid air	10,000 lbs.	4.932

It is apparent from the data that the amount of pressure applied is an important factor. The difference in the osmotic pressure determined from the sap expressed at 10,000 pounds and that expressed at 50,000 is with either method of freezing close to one atmosphere. On the other hand, the method of freezing the tissue previous to extraction of the sap shows but little difference in the osmotic-pressure values obtained. The difference is greater when a pressure of 10,000 pounds is used than when a pressure of 50,000 pounds is employed.

Similar data were obtained with the Iresine in other experiments and also with *Zebrina pendula*.

Comparison of the plasmolytic and cryoscopic methods in determining osmotic pressure. Tables 2 and 3 give the osmotic pressures as determined by the plasmolytic and cryoscopic methods. Table 2 gives the data for *Zebrina pendula*, and table 3 for Iresine. The four series of determinations for each species were made on different days. For the cryoscopic determination, the leaves were frozen by immersion in liquid air and extraction

of the sap was made under a pressure of 50,000 pounds; conditions which would yield the highest values.

TABLE 2. *Zebrina pendula*

No. of Exp.	Osm. Pr. in Atm. with Sucrose	Osm. Pr. in Atm. with $CaCl_2$	Average	Cryoscopic
No. 1.....	4.233	4.480	4.356	5.173
No. 2.....	4.121	4.650	4.385	6.425
No. 3.....	4.188	4.704	4.446	5.714
No. 4.....	4.211	4.569	4.390	5.293

TABLE 3. *Iresine*

No. of Exp.	Osm. Pr. in Atm. with Sucrose	Osm. Pr. in Atm. with $CaCl_2$	Average Atm. Plasmolytic	Atm. Cryoscopic
No. 1.....	6.720	7.078	6.899	8.300
No. 2.....	6.496	7.101	6.798	9.443
No. 3.....	6.944	6.809	6.876	7.981
No. 4.....	6.329	6.918	6.623	8.016

A comparison of the various data indicates that the cells must permit of the entrance of $CaCl_2$ more readily than the sucrose, since the osmotic-pressure values as determined are higher when the plasmolyzing solution is $CaCl_2$ than when the solution is sucrose. The values as determined by the cryoscopic method are in all cases higher than the osmotic-pressure values as obtained from the plasmolytic method. In the case of *Zebrina pendula*, hundreds of determinations made during the past ten years of the osmotic pressures of epidermal cells by the plasmolytic method, using various salts and sugars, give values which range between 4.0 and 4.5 atmospheres. It would appear, therefore, that in the case of *Zebrina pendula* the values as obtained by the plasmolytic method are not far from being the true values for the cells observed. It should be pointed out, however, that the plasmolytic values were obtained entirely from pigmented cells of the lower epidermis, and it is not improbable that the osmotic pressure of the chlorophyllous cells is greater than that of the pigmented cells. Furthermore, the guard cells of the stomates, as pointed out by Iljin (6), may show a much higher osmotic pressure than the adjacent cells, the differences observed being as high as 80 atmospheres. In our laboratory, R. A. Wiggans has found by the plasmolytic method, differences not to exceed 7 atmospheres for Iresine and 6 atmospheres for *Zebrina pendula*.

Another possible explanation to account for the great difference is that by the cryoscopic method the sap is expressed from the conducting tissue as well, and it is not improbable that the concentration of solutes in the conducting system may be in excess of that of the mesophyll cells. This idea is supported by the evidence of Davis, Daish, and Sawyer (1), who found that the ratio of hexoses to sucrose is higher in the midrib and stalks than in the remainder of the leaf, and also that total sugars are actually higher.

Remarks

As pointed out previously, Harvey (5) noted that the method of freezing the tissue affected the concentration of the sap. Assuming that the same pressure was used by him in expressing the sap from leaves frozen by the different methods, it follows that the investigator must exercise some discretion in the method employed for freezing the tissue.

It seems to the writers, however, that the amount of pressure applied is more important than the method of freezing. What amount of pressure should be used would depend upon the character of the plant tissue under investigation. It would seem desirable to use that pressure which would yield a sap of the greatest concentration, and the investigator should state specifically the pressure employed. Furthermore, if tissues of unlike character are under investigation, as, for example, parenchymatous and woody tissues, it would seem that a greater pressure would be needed for the woody tissue than for the parenchymatous tissue in order to give comparable results. In other words, with a parenchymatous tissue a pressure of 10,000 pounds might yield a sap containing 95 percent of the solutes of the original sap, while for a woody tissue the same percentage of solutes might be obtained only with a pressure of 50,000 pounds. It seems to the writers, therefore, that in any investigation involving the determination of osmotic pressures of plant tissues, preliminary experiments are essential in order to determine the most desirable methods of freezing and the amount of pressure to be applied.

Summary

1. A piston-cylinder apparatus is described for use in the expression of cell sap.

2. Recommendation is made with respect to the use of standard materials-testing machinery where definite pressures are available.

3. Experiments show that a pressure of 50,000 pounds yields a more concentrated sap than a pressure of 10,000 pounds.

4. No great differences were found in the concentration of the sap expressed from leaves frozen with liquid air or with an ice-salt mixture.

5. Considerable differences were observed between the osmotic pressure as determined by the plasmolytic and by the cryoscopic methods.

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